

Docket No. 1021.43414X00
Appln. No. 10/759,179
March 29, 2006

AMENDMENTS TO THE CLAIMS:

The following listing of claims replaces all prior listings, and all prior versions, of claims in the application.

LISTING OF CLAIMS:

1. (Currently amended) A method for expressed gene analysis comprising:
_____subjecting a gene to be analyzed to nucleic acid amplification using a forward primer specifically hybridizing to the gene to be analyzed, a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene and comprising a second base sequence closer to the 5' end of the primer than the first base sequence, a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore and at another end with a quencher, reverse transcriptase, RNA polymerase, and ribonuclease H and/or exonuclease;
_____digesting the probe bound to the first base sequence by the ribonuclease H or exonuclease at the time of the nucleic acid amplification; and
_____detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification,
wherein the gene to be analyzed is prepared by ~~introducing~~ the introduction of the first base sequence and the second base sequence comprising a promoter sequence of RNA polymerase, which are nonspecific to the base sequence of the target gene, into the target gene so that the second base sequence is bound to a position closer to the a 5' end of the gene to be analyzed than the first base sequence.

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2. (Currently amended) The method for expressed gene analysis according to claim 1, wherein the gene to be analyzed is cDNA comprising the first base sequence and the second base sequence introduced therein by the introduction with subjecting the mRNA of the target gene to reverse transcription using a-the primer for introduction which comprises the first base sequence, which is closer to the 5' end of the primer than the third base sequence comprising a sequence that specifically hybridizes to the target gene and the second base sequence, which is closer to the 5' end of the primer than the first base sequence.

3. (Original) The method for expressed gene analysis according to claim 1, wherein the nucleic acid amplification is conducted by sequentially repeating the following steps 1) to 3):

- 1) transcription of the gene to be analyzed into RNA with the aid of RNA polymerase;
- 2) reverse transcription of the RNA using the forward primer and the reverse transcriptase or ribonuclease H to synthesize single-stranded cDNA; and
- 3) synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and DNA polymerase.

4. (Original) The method for expressed gene analysis according to claim 1, wherein the nucleic acid amplification is conducted by sequentially repeating the following steps 1) to 3):

- 1) transcription of the gene to be analyzed into RNA with the aid of RNA polymerase;

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- 2) reverse transcription of the RNA using the forward primer and the reverse transcriptase or ribonuclease H to synthesize single-stranded cDNA; and
 - 3) synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and the reverse transcriptase.
5. (Original) The method for expressed gene analysis according to claim 1, wherein the nucleic acid amplification is conducted at a substantially single temperature.
6. (Original) The method for expressed gene analysis according to claim 5, wherein the single temperature is between 37°C and 55°C.
7. (Original) The method for expressed gene analysis according to claim 1, wherein the RNA polymerase is T7 RNA polymerase and the second base sequence comprises the T7 promoter sequence.
8. (Original) The method for expressed gene analysis according to claim 1, wherein two or more target genes are simultaneously detected in a single reaction vessel using two or more types of probes.
9. (Original) The method for expressed gene analysis according to claim 8, wherein the melting temperatures (T_m values) of the two or more types of probes are substantially the same.
10. (Withdrawn) A kit for expressed gene analysis comprising:

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at least one probe comprising a base sequence identical or complementary to a first base sequence, and labeled at one end with a fluorophore and at another end with a quencher,

wherein both the first base sequence and the second base sequence comprising a promoter sequence of RNA polymerase are nonspecific to a base sequence of a target gene.

11. (Withdrawn) The kit for expressed gene analysis according to claim 10 comprising two or more types of probes having substantially the same T_m values.

12. (Withdrawn) The kit for expressed gene analysis according to claim 11, wherein each of the two or more types of probes comprises several module sequences of 3 or 4 bases, both of the terminal bases of each module sequence are identical to each other, and each probe is constituted by rearranging the order of the module sequences having identical terminal bases.

13. (Withdrawn) The kit for expressed gene analysis according to claim 10, wherein the second base sequence comprises the T7 promoter sequence.

14. (New) The method for expressed gene analysis according to claim 1, wherein the probe is a DNA/RNA hybrid strand.